Review of the Rpt3 Genes Encoding Part of the 26S Proteasome Associated with Loci Underlying Disease Resistance in Soybean

Shivani Malik  
*Southern Illinois University Carbondale, mmalikshivani@gmail.com*

Sukesh Bhaumik  
*Southern Illinois University Carbondale*

David A. Lightfoot  
*Southern Illinois University Carbondale, ga4082@siu.edu*

Follow this and additional works at: [http://opensiuc.lib.siu.edu/psas_articles](http://opensiuc.lib.siu.edu/psas_articles)  

**Recommended Citation**  

This Article is brought to you for free and open access by the Department of Plant, Soil, and Agricultural Systems at OpenSIUC. It has been accepted for inclusion in Articles by an authorized administrator of OpenSIUC. For more information, please contact [opensiuc@lib.siu.edu](mailto:opensiuc@lib.siu.edu).
Review of the Rpt3 Genes Encoding Part of the 26S Proteasome Associated with Loci Underlying Disease Resistance in Soybean

Shivani Malik, Sukesh Bhaumik and David A. Lightfoot*

Department of Biochemistry and Molecular Biology, Southern Illinois University, Carbondale IL 62910, USA

Received: February 24, 2012 / Accepted: March 28, 2012

Abstract

The 26S proteasomal complex is a multifunctional proteolytic machinery of the cell. The proteasome plays role in myriad of cellular functions, which have been further diversified by its separable proteolytic and non-proteolytic sub-complexes. Protein quality control and turnover, cell cycle regulation, gene regulation and DNA repair are among the key processes controlled by the proteasome. Disease resistance in plants invokes changes in all the processes controlled by the 26S proteasome. In this review, the potential contribution of genes encoding the proteasome to disease resistance in soybean (Glycine max L. Merr.) was examined.

Keywords: Proteasome; Rpt3; SCN; SDS; QTL; soybean; Glycine max.

Introduction

The 26S proteasome is a versatile, non-lysosomal protein degradation complex of the cell (Glickman et al., 1998; Voges et al., 1999; Coux, 2002). It consists of two sub-complexes: the 20S catalytic particle (CP) and the 19S regulatory particle (RP). The 19S RP in turn consists of a “base” and a “lid”. The 20S CP has a hollow cylinder like structure consisting of a stack of two alpha and two beta rings in the α7-β7-β7-α7 order. Three of the seven β subunits are catalytically active and possess chymotrypsin-like (β5), trypsin-like (β2) and caspase-like activities (β1) (Dick et al., 1998; Groll and Clausen, 2003). The base of 19S RP consists of a ring of six ATPases (Rpt1-6) and three non-ATPases (Rpn1, Rpn2 and Rpn13) (Glickman et al., 1998; Voges et al., 1999; Coux, 2002; Dick et al., 1998; Groll and Clausen, 2003; Horwitz et al., 2007). The 19S base possesses molecular chaperonin activity that enables it to fold and unfold proteins (Braun et al., 1999). A chain of four or more polyubiquitin moieties marks the protein destined to be degraded (Chau et al., 1989; Finley et al., 1994; Wang et al., 2006). The ubiquitin receptor (Rpn10) in the 19S lid binds to this polyubiquitinated protein (Deveraux et al., 1994; Elsasser et al., 2004) which leads to its unfolding by the 19S base by ATP hydrolysis. The unfolded protein is fed into the 20S CP where the beta subunits proteolyze it (Larsen and Finley, 1994; Navon and Goldberg, 2001; Verma et al., 2004; Guterman and Glickman, 2004; Hanna et al., 2006; Seong et al., 2007a,b; Shreiner et al., 2008; Husnjak et al., 2008). Through this mechanism, the proteasome regulates...
abundance and localization of a large number of transcription factors influencing several cellular processes (Hilt and Wolf, 1996; He et al., 1998). It also degrades oncoproteins, cell cycle dependent cyclins, cyclin dependent kinase inhibitors, thus influencing multitude of cellular processes directly or indirectly. In addition to this proteolytic role, the proteasome has been implicated to regulate transcriptional activation in a proteolytic-independent manner (Collins and Tansey, 2006; Bhaumic and Malik, 2008; Ferry et al., 2009; Lassot et al., 2007). It serves to recruit transcriptional co-activators, chromatin remodeling complexes and enables assembly of the pre-initiation complex at gene promoters to activate transcription. Thus, the proteasome serves as a central hub orchestrating several regulatory mechanisms (Figure 1). This makes it a good candidate for analysis of its contribution, if any, to some of pathological states of plants (in this study soybean).

Analysis in Soybean

Soybean [Glycine max (L.) Merr.] is the world’s most important legume crop grown for its protein and oil content (Messina et al., 1997). Nematode attack (by Heterodera glycines I.) by far has the most damaging effect on soybean crop leading to huge economic losses. Soybean cyst nematode (SCN) has been counteracted by non-host crop rotation practices, use of nematicides and development of resistant cultivars. Use of resistant varieties is a key strategy in SCN management. Although, about 130 sources of SCN resistance have been identified within the Glycine sp., and more than 30 resistance loci identified, not much is clearly known about the underlying SCN resistance genes (Afzal et al., 2008). SCN resistance is controlled by several genes at multiple quantitative trait loci (QTL) (Concibido et al., 2004) and identification of genes contributing to these loci will enable better management of SCN. Fine mapping analysis of SCN resistance loci has revealed two major loci controlling resistance to SCN HG type 0 (Race 3) in soybean cv. ‘Forrest’ (Meksem et al., 2001). One is the Rhg1 locus on linkage group G and other is Rhg4 on linkage group A2. Major loci were also found on linkage groups B1 and D2 in many Plant Introductions (PIs; Afzal et al., 2009; Yue et al., 2001; Webb et al., 1995).

Proteomic studies have shown that among the proteins increased in SCN resistant Rhg1 near isogenic lines (NILs) was a proteasomal component (Afzal et al., 2009; Figure 1). It is interesting to note that proteasome forms an important hub in the SCN

Figure 1. A part of the predicted interactome for soybean showing six proteins from the 2D gel analysis of (32). The multi catalytic endopeptidase shown in blue is the beta-3 subunit of the 20 S proteasome core protein. The proteins encoded by the Rpt3 genes of the 19S regulatory particle are represented by a single pink spot (arrowed). The 14 proteins of the 20S particle and 9 proteins of the 19 S regulatory particle interact to form the 26S proteasome. Also shown in color were proteins changed in abundance by SCN infestation of roots; the thaumatin like protein, cytosolic heat shock protein and triose phosphate isomerase.
resistant protein interactome web with at least 21 interacting partners. Perhaps variations in the proteasome contribute to the SCN QTL. Soybean behaves like a diploidized-tetraploid and homology searches using BLAST showed there were two or four copies of each of the six ATPases subunits. Of these, Rpt3 had two copies on chromosomes 11 (linkage group B1) and 18 (linkage group G). It is intriguing that the copy of same ATPase was conserved on two chromosomal locations. On chromosome 11, Rpt3 maps to a position of 32,631,398-32,634,285. This location overlaps with Satt415 (position: 32,627,233 -32,627,528), a marker in the region mapped to SCN QTL (Figure 2a). This suggests that Rpt3 gene might be associated with SCN QTL. The other copy of Rpt3 lies on linkage group G (chromosome 18) at position 4,388,793-4,389,751. Satt688 (3,264,226-3,264,398) and Satt130 (4,619,181-4,619,599) are within 2 Mbp of Rpt3 on chromosome 18 (Figure 2b). Both Satt688 and Satt130 are associated with SCN QTL. SCN QTL associated with Satt688 were 29-1, 29-4, 29-8 while Satt130 is linked to SCN QTL.
QTL 17-4, 18-5 and 19-4. On linkage group G there was also a QTL for resistance to sudden death syndrome that overlaps the Rps3 genes and the SCN QTL.

An interesting feature of the Rpt3 gene on both the chromosomes was a close conservation of the flanking genes (Figure 3). Conservation of two copies of Rpt3 with a set of few common genes suggests that this genetic unit may be playing important physiological roles in soybean. Serine threonine protein kinases, E2 ubiquitin lyases, harpin induced proteins, Kip1 like proteins and DEAD box helicases were all clustered with the Rpt3 genes at both loci.

The proteasomal component was increased during pathogen infestations probably to degrade the oxidized or damaged proteins or as a defense against oxidative stress posed by SCN feeding (Giulivi et al., 1994; Lee et al., 2006). Mapping of proteasomal genes close to SCN QTL underscores the contribution of proteasome in SCN resistant reaction. Thus, it might serve as an important gene in contributing to resistance to SCN. The proteasome has an interesting architecture, which couples to its exquisite regulation. It has a base of six ATPases, which sits atop the 20S cylinder. Upon ATP binding, the C-terminal hydrophobic tyrosine-X-motif of the base ATPases docks into the α subunits of the 20S core. ATP hydrolysis by the base then serves to open the gate to proteolytic 20S core to feed the proteins into the 20S barrel (Smith et al., 2005). Previous work (Afzal et al., 2009) and mapping of both the 20S and 19S components to SCN QTL indicates that a part of its role is SCN resistance is through its proteolytic activity. The 26S proteasome recognizes proteins to be degraded by a polyubiquitin chain of four or more ubiquitin moieties. The ubiquitin chain on a cellular protein is formed by isopeptide bond formation between C-terminus of ubiquitin and ε-amino lysine side chain of the target protein or another ubiquitin molecule (Pickart and Eddins, 2004; Pickart and Fushman, 2004; Wang et al., 2006) by the sequential action of E1 activating, E2 conjugating and E3 ligating enzymes. Nature of the lysine linkage is a key factor determining the fate of the protein. K-48 linked poly ubiquitin chains are targeted for degradation while K-68 mostly serves in signaling pathways (Wang et al., 2006; Chau et al., 1989; Finley et al., 1994).

In addition, the 19S base ATPases can exist as an APIS (AAA ATPases independent of 20S) complex separate from the 20S core (Sun et al., 2002). This complex plays a crucial role in regulation of transcriptional activation in yeast and humans (Losso et al., 2007; Gonzalez et al., 2002; Lee et al., 2005; Bhat et al., 2008; Malik et al., 2009; Truax et al., 2010). Thus, 19S ATPases might also function to regulate transcription of other genes contributing to SCN reaction. The 19S ATPases have been shown to play key roles in histone covalent modification in yeast and mammals (Lee et al., 2005; Ezkova and Tansey, 2004; Laribee et al., 2007; Kinyamu et al., 2008; Kouses et al., 2008, 2009). In yeast, inactivation of Rpt6/Sug1 leads to decreased dimethylated histone H3 lysine 4 (H3K4 di-me) and acetylated histone H3 (Lee et al., 2005). H3K4 methylation is associated primarily with gene activation but also contributes to gene silencing of mating type locus. Acetylation of H3 is a strong gene activating signal serving to remodel promoter chromatin. These modifications also serve as a binding sites for effector proteins which perform their designated functions (Strahl and Allis, 2000; Sims and Reinberg, 2006) allowing the proteasome to exercise widespread control over various pathways. The 19S base also has chaperonin like activity and might also be serving to play some role in SCN resistance consistent with increased abundance of chaperonins in SCN resistant NIL roots (Afzal et al., 2009).
Concluding Remarks

The proteasome with its far-ranging functions is an attractive candidate gene for regulating diseased states in plants. Bioinformatics analyses based on protein abundances, protein interactions and the location of genes encoding those proteins has inferred the proteasome may be contributing to SCN resistance in soybean. This link however, needs to be confirmed using other approaches. These approaches in soybean include candidate gene TILLING, EcoTILLING and complementation analysis, as has been performed for soybean cv 'Forrest' (Liu et al., 2010). Evaluation of the role of the proteasome through expression studies and association analysis may also yield interesting information about its mechanism of action. Identification of candidate genes underlying such diseases will provide a better insight of causal factors of such diseases and will contribute to development of better ways to manage them.

References


